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MINIREVIEW

The Novel Human Coronaviruses NL63 and HKU1[▽]

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In the mid-sixties of the previous century, the first two human coronaviruses (HCoV) were identified: HCoV-229E and HCoV-OC43 (29, 50, 70). These two human coronaviruses were studied extensively from approximately 1965 to the mid-1980s (7, 37, 49, 50, 57, 70). HCoV-229E is a member of the group I coronaviruses, and HCoV-OC43 is a member of group II. Besides the human coronaviruses, there are several group I and group II animal coronaviruses that infect cattle, pigs, cats, dogs, mice, and other animals (33). There is one additional branch, the group III coronaviruses, which are found exclusively in birds (33).

By infecting healthy volunteers, researchers learned that infection with HCoV-229E or HCoV-OC43 results in a common cold (7, 8, 29), and since then, HCoVs have been considered to be relatively harmless respiratory pathogens. This image was roughly disturbed when severe acute respiratory syndrome (SARS)-CoV was introduced into the human population in the winter of 2002 to 2003 in China. SARS-CoV causes a severe respiratory illness with high morbidity and mortality (16, 40). The virus originated from a wild-animal reservoir, most likely bats (43, 46), and was transmitted to humans via infected civet cats. The epidemic was halted in 2003 by a highly effective global public health response, and SARS-CoV is not currently circulating in humans. However, the SARS outbreak brought coronaviruses back into the limelight, and a renewed interest in this virus family resulted in the identification of two more human coronaviruses. We discovered HCoV-NL63, a novel member of group I, in a child with bronchiolitis in The Netherlands (74). HCoV-HKU1, a novel group II virus from an adult with chronic pulmonary disease in Hong Kong, was described in 2005 (81). An animal model is currently lacking for these previously unknown viruses. Nevertheless, since the discovery of HCoV-NL63 and HCoV-HKU1, much knowledge about these viruses has been gained. Several groups have studied their worldwide spread, association with human disease, replication characteristics, genome organization, and genetic diversity.

CORONAVIRUS GENOME ARCHITECTURE

Coronaviruses are positive-stranded RNA viruses, with the largest viral genome of the RNA viruses (27 to 33 kb) (reviewed in reference 47). The single-stranded genome is capped and polyadenylated. The virus particle is enveloped and carries extended spike proteins on the membrane surface, providing the typical crown-like structure (crown = corona) seen by electron microscopy. The coronaviruses share a conserved organization of their RNA genomes (Fig. 1). The 5′ two-thirds of the genome contains the large 1a and 1b open reading frames (ORFs) encoding the proteins necessary for RNA replication (the nonstructural proteins), whereas the 3′ one-third contains the ORFs coding for the structural proteins: hemagglutinin esterase (HE) (only for group IIa), spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N) (27, 56, 62). Interspersed among the structural protein genes one can find accessory protein genes that differ among species in number and position.

A common transcription regulatory sequence (TRS) is located upstream of the 5′ end of each ORF. This TRS sequence is essential for the formation of subgenomic mRNAs. The subgenomic mRNAs are generated by discontinuous minus-strand synthesis and are copied into plus-strand mRNAs, which all have a common leader sequence of about 70 nucleotides at their 5′ ends that is identical to the sequence at the 5′ end of the genomic RNA. Each subgenomic mRNA and the viral genomic RNA, which also serves as an mRNA, is translated to yield, in most cases, only a single protein encoded by the ORF located immediately downstream of the TRS sequence (27, 42, 67). Some subgenomic mRNAs are bicistronic, and in these cases, translation of a second ORF depends on an internal ribosomal entry site (68).

The replicase proteins are synthesized from the partially overlapping ORF1a and ORF1b, and ribosomal access to ORF1b is regulated by a hairpin-pseudoknot structure that acts as a ribosomal frameshifting element (RFS) (52). The coronavirus ORF1ab is translated as a single polyprotein that is cotranslationally cleaved by two proteases encoded in the 5′ region of the 1ab protein gene. A papain-like protease is encoded by the nonstructural protein 3 (*nsp3*) gene located near the 5′ terminus of the genome. This protease consists of two domains: $PL1^{pro}$ and $PL2^{pro}$. The *nsp5* gene encodes the second protease of coronaviruses. It has a predicted serine-like protease activity and is designated the "main" protease (M^{pro}) to stress its dominant function. This protease processes the majority of cleavage sites between the protein domains of the 1ab polyprotein (86).

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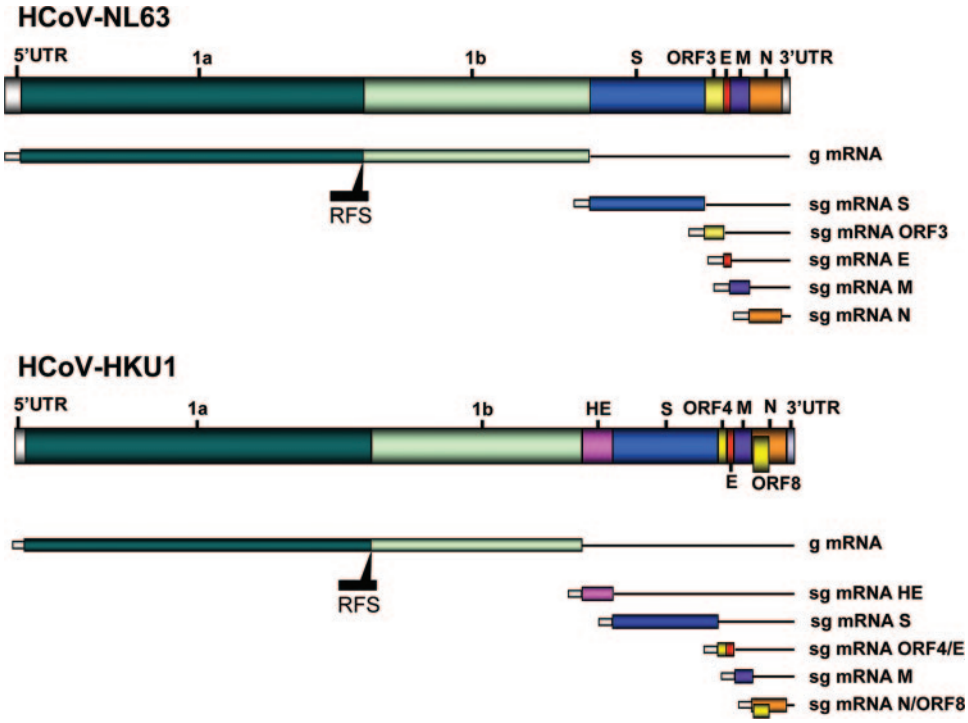

FIG. 1. Schematic organization of the HCoV-NL63 and HCoV-HKU1 genomes. All ORFs and the 3′ and 5′ untranslated regions (UTR) are marked. Below the scheme, the corresponding subgenomic (sg) mRNAs generated during the discontinuous transcription process are shown (putative for HCoV-HKU1). RFS represents the putative ribosomal frameshifting element between the 1a and 1b genes.

GENOME ORGANIZATION OF HCoV-NL63 AND HCoV-HKU1

The RNA genome of HCoV-NL63 is 27,553 nucleotides, with a poly(A) tail (Fig. 1). With a GC content of 34%, HCoV-NL63 has one of the lowest GC contents of the coronaviruses, for which GC content ranges from 32 to 42% (74). Untranslated regions of 286 and 287 nucleotides are present at the 5′ and 3′ termini, respectively.

Genes predicted to encode the S, E, M, and N proteins are found in the 3′ part of the HCoV-NL63 genome. The HE gene, which is present in some group II coronaviruses, is absent, and there is only a single, monocistronic accessory protein ORF (ORF3) located between the S and E genes. Subgenomic mRNAs are generated for all ORFs (S, ORF3, E, M, and N), and the core sequence of the TRS of HCoV-NL63 is defined as AACUAAA (56). This sequence is situated upstream of every ORF except for the E ORF, which contains the suboptimal core sequence AACUAUA. Interestingly, a 13-nucleotide sequence with perfect homology to the leader sequence is situated upstream of the suboptimal E TRS. Annealing of this 13-nucleotide sequence to the leader sequence may act as a compensatory mechanism for the disturbed leader-TRS/body-TRS interaction (56).

The 1a/1b ORF of HCoV-NL63 contains a potential elaborated pseudoknot structure that triggers a −1 ribosomal frameshift at position 12439 to translate the complete 1ab polyprotein (54). The *nsp3* and *nsp5* genes of HCoV-NL63 encode the two putative viral proteases, papain-like protease and M^{pro}, respectively. The cleavage sites recognized by these enzymes are predicted to be essentially identical to those of the other coronaviruses, with one exception. At the *nsp13*/*nsp14* junction, the cleavage site contains a histidine at position P1 instead of the regular glutamine (54). This altered cleavage site is present not only in laboratory strains of HCoV-NL63 (strain Amsterdam 1 and strain NL) but also in two clinical isolates (Amsterdam 57 and Amsterdam 496). It has not been formally demonstrated that this noncanonical cleavage site is indeed functional.

The genome of HCoV-HKU1 is a 29,926-nucleotide, polyadenylated RNA (81) (Fig. 1). The GC content is 32%, the lowest among all known coronaviruses. The genome organization is the same as that of other group II coronaviruses, with the characteristic gene order 1a, 1b, HE, S, E, M, and N. Furthermore, accessory protein genes are present between the S and E genes (ORF4) and at the position of the N gene (ORF8). The TRS is presumably located within the AAUC UAAAC sequence, which precedes each ORF except E. As in sialodacryoadenitis virus and mouse hepatitis virus (MHV), translation of the E protein possibly occurs via an internal ribosomal entry site. The 3′ untranslated region contains a predicted stem-loop structure immediately downstream of the N ORF (nucleotide position 29647 to 29711) (81). Further downstream, a pseudoknot structure is present at nucleotide position 29708 to 29760. Both RNA structures are conserved in group II coronaviruses and are critical for virus replication (26, 78).

The large 1ab polyprotein of HCoV-HKU1 is synthesized via a −1 ribosomal frameshift at a putative pseudoknot structure (position 13594). In the N terminus of *nsp3* (containing $PL1^{pro}$ and $PL2^{pro}$), tandem copies of a 30-base repeat encod-

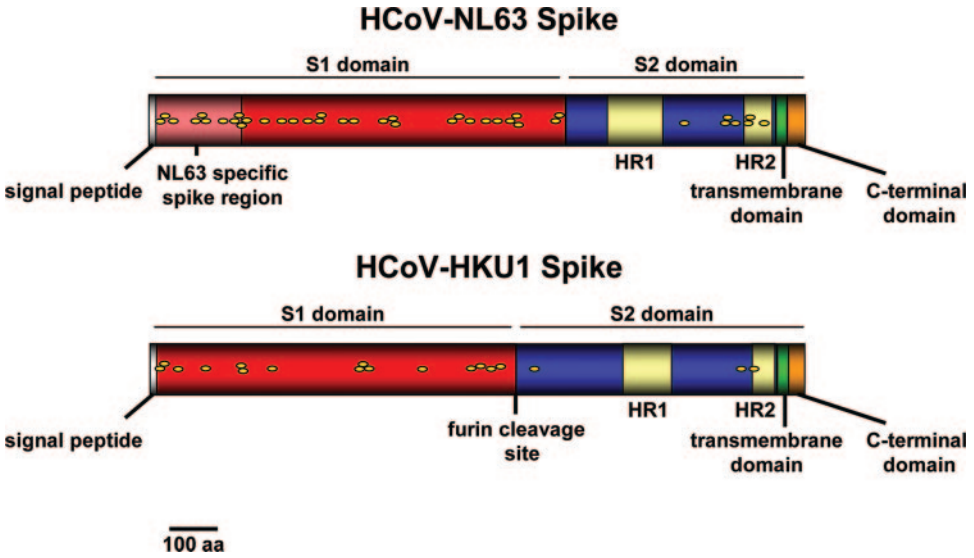

FIG. 2. Schematic representation of the spike protein of HCoV-NL63 and HCoV-HKU1. The signal peptide was identified by using the SignalIP 3.0 server (4, 53). The border between the S1 and S2 domains corresponds to the predicted furin-like enzyme cleavage site identified with the ProP 1.0 server (17). The yellow dots represent N-glycosylation sites predicted with the NetNGlyc1.0 server (R. Gupta, E. Jung, and S. Brunak, unpublished data). Heptad repeat regions (HR1 and HR2) were identified by using protein alignments. The transmembrane domain was identified with the TMHMM 2.0 server (39, 66).

ing NDDEDVVTGD are present, followed by 30-base regions that encode NNDEEIVTGD and NDDQIVVTGD located within the acidic domain upstream of PL1^{pro}. These acidic repeats are not observed in other coronaviruses, and their function is still unknown. The numbers of repeats vary among HCoV-HKU1 isolates (71, 83).

The characteristic amino acid sequences required for proteolytic cleavage of the HCoV-HKU1 1ab polyprotein by the papain-like protease and M^{pro} are present. However, it has been suggested that HCoV-HKU1 shares the unusual cleavage site at *nsp13*/*nsp14* with HCoV-NL63 (80). When the *nsp13*/*nsp14* cleavage site of HCoV-HKU1 is aligned with those of other group II coronaviruses, the P1 position is occupied by a histidine instead of the regular glutamine. This is the same alignment as described for HCoV-NL63 (54). However, HCoV-HKU1 also has a standard cleavage site with the regular glutamine at the P1 position for *nsp13*/*nsp14*. This standard site is recognized by Zcurve_CoV 2.0 software (24) and is located 24 residues amino terminally of the unusual histidine-containing cleavage site. Future experiments should resolve which site is cleaved by HKU1-M^{pro}.

CORONAVIRUS FUSION PROTEIN

The coronavirus spike protein is a membrane-anchored glycoprotein that is found on the virion surface in a trimeric form. The N-terminal half of the protein (S1) contains the receptor-binding domain, and the C-terminal half (S2) is the membrane-anchored domain with fusogenic activity (Fig. 2). Similar to those of other class I fusion proteins, the S2 domain contains two heptad repeat (HR) regions, of which one (HR2) is located close to the transmembrane anchor. Class I fusion proteins carry a hydrophobic fusion peptide at or close to the N terminus of the membrane fusion subunit. For SARS-CoV, a fusion peptide was recognized at the N terminus of the S2 subunit (59). Thus, the spike protein displays strong similarities to class I virus fusion proteins, but there is one unusual characteristic. It does not require cleavage into S1 and S2 for coronavirus infection, unlike other class I fusion proteins (14).

Coronavirus entry into the target cell is facilitated either by direct fusion with the plasma membrane or by endocytosis and subsequent fusion to the endosomal membrane. Viruses that use pH-dependent endocytosis are sensitive to treatment with lysosomotropic agents that lower the endosomal pH. SARS-CoV and HCoV-229E are examples of coronaviruses that use endosomal entry (5, 30, 63). Treatment with lysosomotropic agents like bafilomycin A, chloroquine, and NH_4Cl inhibits entry of these viruses.

SPIKE PROTEIN OF HCoV-NL63 AND HCoV-HKU1

The N-terminal part of the S protein of HCoV-NL63 contains a unique 179-amino-acid domain that is not present in other coronaviruses, including its closest relative, HCoV-229E. This domain of the HCoV-NL63 S protein contains several potential glycosylation sites (Fig. 2) and, in fact, represents the most-variable region of the complete HCoV-NL63 genome, suggesting a role in immune evasion (73). On the other hand, strains of MHV that differ in virulence and tissue tropism have deletions of different sizes in the N-terminal domain of S (58). Furthermore, the difference between transmissible gastroenteritis virus (TGEV; an enterotropic porcine virus) and porcine respiratory coronavirus (PRCoV; a respiratory porcine coronavirus) is also pinpointed to a 200-amino-acid deletion in the N-terminal region of S (77). Thus, the N-terminal region of NL63-S might also be a determinant of cell tropism and the pathogenicity of the virus.

Some group II and group III coronaviruses possess a furin-specific cleavage site between the S1 and S2 domains. A furin cleavage site is lacking in HCoV-NL63. This is not unusual, as

none of the group I coronaviruses harbors a furin cleavage site between the S1 and S2 domains. The S1 domain contains the receptor-binding region (see Cell Tropism and Receptor Usage, below), and the S2 domain is involved in fusion of viral and cellular membranes. The S2 domain contains two heptad repeat regions: HR1 (residues 949 to 1064) and HR2 (residues 1234 to 1293). As in the other group I coronaviruses, the NL63-HRs are longer than those of group II or III coronaviruses (Fig. 2) (6). Both HRs of HCoV-NL63 contain a 14-amino-acid insert (two extra helical turns). After binding of a virus to its target cell, the HR regions change their conformation, which is required for membrane fusion. The HR1 and HR2 peptides eventually assemble into the hexameric form, the very stable six-helix bundle (55). An NL63-HR2 peptide can efficiently block HCoV-NL63 infection, which confirms that HR1 and HR2 interactions are vital during HCoV-NL63 replication (55).

It is not very clear whether HCoV-NL63 entry occurs via fusion with the plasma membrane or via endocytosis and subsequent fusion with the endosomal membrane. HCoV-NL63 entry is not very sensitive to lysosomotropic agents (32, 34). Incubation with bafilomycin A only mildly affects HCoV-NL63 S-mediated entry, indicating that internalization of HCoV-NL63 into its host cell is less dependent on low pH than are SARS-CoV and HCoV-229E entry. It is possible that HCoV-NL63 uses the endosomes but is not strictly dependent on pH-dependent cleavage or that fusion can occur via the plasma membrane.

The S protein of HCoV-HKU1 does contain a potential furin cleavage site between the S1 and S2 domains: RRKRR, with cleavage predicted to occur between residues 760 and 761 (Fig. 2) (81). The S2 domain contains two heptad repeats, located at residues 982 to 1083 (HR1) and 1250 to 1297 (HR2), and the S1 domain presumably contains the receptor-binding site. However, which cellular surface molecule is used as a receptor by HCoV-HKU1 is currently unknown.

CELL TROPISM AND RECEPTOR USAGE

HCoV-NL63 can be cultured in monkey epithelial cell lines (LLC-MK2, Vero E6, or Vero B4) (23, 60, 74). This preference for monkey epithelial cells is shared with SARS-CoV, but the closest relative of HCoV-NL63, HCoV-229E, cannot replicate on these cells. It was generally thought that all group I coronaviruses use CD13, also known as aminopeptidase N, as a receptor. CD13 usage has been described for HCoV-229E, porcine coronaviruses TGEV and PRCoV, and the feline and canine coronaviruses (15, 69, 85). Surprisingly, HCoV-NL63 is not able to use CD13 as a receptor for cell entry (31). A detailed analysis of cell tropism revealed that certain human cells, namely Huh-7 cells (a human hepatocellular carcinoma cell line), can also be infected by HCoV-NL63 (31). This underscores the similarity of HCoV-NL63 and SARS-CoV, which can readily infect Huh-7 cells. This shared cell tropism is suggestive of shared receptor usage. SARS-CoV is unique among the coronaviruses in that it uses angiotensin-converting enzyme 2 (ACE2) to bind and enter target cells (45). ACE2 is a surface molecule that was not previously known as a receptor for coronaviruses. Hofmann et al. demonstrated that HCoV-NL63 is the only other coronavirus that uses ACE2 as an entry receptor (31, 32). There is no similarity between the HCoV-NL63 and SARS-CoV spike proteins that could explain this shared receptor usage (45). The interaction between HCoV-NL63 and ACE2 is probably different from the interaction between SARS-CoV and ACE2. Mutations in ACE2 known to affect binding by SARS-CoV do not affect binding by HCoV-NL63 (32). Furthermore, the receptor binding domain of HCoV-NL63, which is located between residues 232 and 694 in the S protein, is not linear, whereas the SARS-CoV receptor binding domain is linear (79). Correct folding of the S protein is probably needed for HCoV-NL63-S to bind the ACE2 molecule (32).

The ACE2 molecule is a surface molecule that is localized to arterial and venous endothelial cells, arterial smooth muscle cells, and epithelia of the small intestine, as well as to ciliated tracheobronchial airway epithelia, thus supporting virus infection in the airways (28, 64). ACE2 is a homologue of the ACE protein, and both are key enzymes of the renin-angiotensin system. ACE activates the renin-angiotensin system by cleaving angiotensin I into angiotensin II, whereas ACE2 negatively regulates this system by inactivating angiotensin II. The renin-angiotensin system has a role in severe acute lung injury (35), with ACE2 playing a protective role in lung failure and its counterpart ACE promoting lung edema and impaired lung function during acute lung injury.

Interestingly, infection with SARS-CoV suppresses the expression of ACE2 protein, and it is hypothesized that the reduced level of ACE2 during SARS-CoV infection is the main cause of the severe pneumonia and acute, often lethal, lung failure associated with this virus (41). It is important to compare the pathogenicity of SARS-CoV and HCoV-NL63 with respect to ACE2 binding and downregulation. The reduced pathogenicity of HCoV-NL63 suggests that ACE2 receptor usage by the virus is not the only factor that determines the severity of viral pathogenicity. Further research on this subject, especially on the modulation of ACE2 expression levels during HCoV-NL63 infection, is warranted in order to understand the difference in lung pathogenicity of SARS-CoV and HCoV-NL63.

It has not been possible to culture HCoV-HKU1, despite attempts to do so in the following cell lines: RD (human rhabdomyosarcoma), I13.35 (murine macrophage), L929 (murine fibroblast), HRT-18 (colorectal adenocarcinoma), B95a (marmoset B-lymphoblastoid), mixed neuron-glia culture, MDCK (canine kidney), LLC-MK2 (rhesus monkey kidney), HEp-2 (human epithelial carcinoma), MRC-5 (human lung fibroblast), FRhK-4 (rhesus monkey kidney), A-549 (lung epithelial adenocarcinoma), BSC-1 (African green monkey kidney), CaCO2 (human colorectal adenocarcinoma), Huh-7 (human hepatoma), and Vero E6 (African green monkey kidney) (81).

DISEASES ASSOCIATED WITH HCoV-NL63 AND HCoV-HKU1 INFECTIONS

After the discovery of HCoV-NL63 and HCoV-HKU1, several groups reported infections by these viruses in different countries, illustrating that these viruses have spread worldwide (1, 2, 12, 13, 18, 21, 25, 36, 51, 65, 71, 72). The viruses can be detected in 1 to 10% of patients with acute respiratory tract

infections, and double infections with other respiratory viruses are common (73). The first described cases of HCoV-NL63 infections were in young children with severe lower respiratory tract infections (LRTIs) in hospital settings (23, 74). Another study reported one HCoV-NL63-infected elderly Canadian patient who died 5 days after the onset of disease (2), showing that the severity of the respiratory disease can be substantial. On the other hand, several of the HCoV-NL63-infected patients showed relatively mild symptoms like fever, cough, sore throat, and rhinitis (2).

A clear link between HCoV-NL63 and respiratory diseases was established in the German prospective population-based study on LRTI in children less than 3 years of age (22, 38). Of the children with HCoV-NL63 infections, 45% had laryngotracheitis (croup) compared to only 6% in the control group. Multivariate analysis demonstrated that the chance of croup is 6.6 times higher in HCoV-NL63-positive children than in HCoV-NL63-negative children (75). Croup is a common manifestation of LRTIs in children. The cause was generally assumed to be a respiratory virus, e.g., one of the parainfluenza viruses, but now it is apparent that HCoV-NL63 also plays a major role in this disease.

HCoV-NL63 has also been associated with Kawasaki disease (20). Kawasaki disease is one of the most common forms of childhood vasculitis (10). It presents with prolonged fever and a polymorphic exanthem, oropharyngeal erythema, and bilateral conjunctivitis. A number of epidemiological and clinical observations previously suggested that an infectious agent might be the cause of Kawasaki disease (reviewed in reference 9). That there may be a link between HCoV-NL63 and Kawasaki disease is fascinating, but multiple groups recently questioned this association. These groups screened for HCoV-NL63 in respiratory material from Kawasaki disease patients, but none were able to confirm the link between HCoV-NL63 and Kawasaki disease (3, 11, 19, 61).

The first described HCoV-HKU1 cases were among elderly patients with major underlying diseases, in particular of the respiratory and cardiovascular systems. Of 10 patients with HCoV-HKU1 infections, 2 patients (aged 66 and 74), both with another serious underlying disease, died (82). Another Hong Kong study described 11 infected children, all with upper or lower respiratory tract infections, of which the majority had an underlying illness (8 of 11). A third Hong Kong study identified 13 HKU1-infected children with acute respiratory tract infections (44). Of these, eight had an underlying illness. In the United States, HCoV-HKU1 was identified in nine children with upper or lower respiratory tract infections; five of them had an underlying disease (21). In France, three of six HCoV-HKU1 infections were observed in patients with an underlying disease (71). These studies suggest that HCoV-HKU1 infection may aggravate the conditions of persons with an underlying disease, thus requiring hospitalization.

The respiratory symptoms accompanying a HCoV-HKU1 infection are usually rhinorrhea, fever, coughing, and wheezing, and disease manifestations include bronchiolitis and pneumonia (44, 65), but Vabret et al. suggested that HCoV-HKU1 might also be involved in gastrointestinal disease (71). They described six HCoV-HKU1 infections in France, of which the majority included upper respiratory tract illness, but three patients were admitted to the hospital because of acute enteric disease. The fact that HCoV-HKU1 was detected in stool samples from two of the patients supports the idea that this virus may also play a role in gastrointestinal disease. A large study of children with acute respiratory tract infections demonstrated an interesting association between HCoV-HKU1 and febrile seizures (44). These symptoms were more frequently observed in HCoV-HKU1-infected children than in HCoV-OC43-infected children.

HCoV-HKU1 and HCoV-NL63 infections are generally not life threatening, certainly not in otherwise healthy persons. This suggests that HCoV-NL63 and HCoV-HKU1, like HCoV-229E and HCoV-OC43, are common cold viruses (48) that can cause more-severe clinical symptoms in young children, elderly persons, and those that are immunocompromised (49, 76).

VARIABILITY

Clinical HCoV-NL63 isolates display sequence heterogeneity, and phylogenetic analyses revealed that there are two genotypic subgroups (1, 12, 51, 72, 74). This was first demonstrated by analyzing a part of the 1a ORF, and S ORF sequences have confirmed the presence of two genotypes. Surprisingly, typing based on the 1a ORF does not match S-based typing for some isolates (51), suggesting that recombinant forms circulate. Sequencing of the full-length genomes of two additional clinical isolates demonstrated that the HCoV-NL63 genome is a mosaic structure comprised of fragments of two donor sequences (56a).

The genetic variability among HCoV-HKU1 isolates suggests that this virus was introduced into the human population some time ago. The sequences of HCoV-HKU1 isolates display marked genetic variability. In one study in Hong Kong, the S and N ORF sequences from nine patients were analyzed. The majority of isolates ($n = 7$) belonged to one genotype (genotype A), and the other two isolates formed a separate subgroup (genotype B) (82). HCoV-HKU1 isolates from Australia belong to one genotype ($n = 8$) (65). Woo et al. investigated whether recombinant forms of genotype A and genotype B circulate (83). Full-genome sequencing of 22 HCoV-HKU1 isolates illustrated that, besides genotype A and genotype B, there is another strain (genotype C) that is the resultant of recombination between genotypes A and B (83).

CONCLUDING REMARKS

The renewed interest in coronaviruses since the outbreak of SARS-CoV has revealed that at least four human coronaviruses circulate worldwide, generally causing relatively mild respiratory symptoms. However, in young children, immunocompromised patients, or otherwise-weakened persons, these viruses can cause more-serious respiratory tract disease that requires hospitalization.

The ACE2 molecule, which is used as a receptor by HCoV-NL63 and SARS-CoV, has recently been associated with respiratory disease. The fact that ACE2 is also involved in protection against lung damage is intriguing and suggests opportunities to treat complications during respiratory tract infections. The fact that SARS-CoV and HCoV-NL63 use the same receptor, while their pathogenicities and disease courses

are utterly different, calls for detailed comparisons of the immune responses, inflammation processes, and in vivo replication characteristics of these two viruses.

The identification and characterization of novel respiratory viruses is of obvious clinical importance. Diagnostics can be improved, and new therapies can be developed. An antiviral strategy that would target all human coronaviruses, e.g., broad-spectrum inhibitors of the M^{pro} enzymes (84), is promising. For HCoV-NL63, several compounds that efficiently inhibit distinct steps of the viral replication cycle have been described (55). A short interfering RNA (siRNA) targeting the S gene of HCoV-NL63 is one of these potent inhibitors. Inhalation of a cocktail of siRNAs targeting all the different coronaviruses or perhaps all respiratory viruses may be an effective and simple therapy to block viral replication in the lungs.

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